Restriction Requirement

Claim 1 has been amended to incorporate using soluble IL-1 receptor in compliance with the Restriction Requirement. Applicants reserve the right to file a separate application directed to the canceled subject matter.

Rejection Under 35 U.S.C. § 112, First Paragraph

Claims 1-4, 12, 15, 17, 19-23, 25, 26 and 45 have been rejected under 35 U.S.C. § 112, First Paragraph, as allegedly failing to comply with the enablement requirement of the written description for the rejected claims. Applicants traverse this rejection. Reconsideration and withdrawal thereof are respectfully requested.

The Examiner has indicated subject matter which would overcome this rejection at page 3, para. 11 in the Office action, which states that the application is "enabling for a method of treating a [sic] arthritis in a mammalian host, generating a recombinant viral vector comprising a DNA sequence encoding soluble IL-1 receptor operatively linked to a promoter, infecting *in vitro* a population of autologous cultured synovial cells with said recombinant viral vector resulting in a population of transduced synovial cells and transplanting said transduced synovial cells by intraarticular injection to an arthritic joint space of a mammalian host, such that expression of said DNA sequence in said joint space results in a reduction of cartilage destruction or a reduction in synovitis."

The present claims recite this language. Accordingly, this rejection has been overcome.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 1-4, 12, 15, 17, 19-23, 25, 26 and 45 have been rejected under 35 U.S.C. § 112, Second Paragraph, as being indefinite. Applicants traverse this rejection.

The Examiner objects to language such as "transducing a population of target cells" because it lacks clarity. The presently amended claim 1 does not recite this language. Further, the Examiner has objected to the use of abbreviations "MFG" and "sIl-1r type" and so on in claims 22 and 23. The presently amended claim 23 does not recite this abbreviation. As for "MFG", Applicants note that "MFG" is not an abbreviation but rather the actual name of the viral vector discussed in the application. Thus, this rejection has been overcome.

Rejection Under 35 U.S.C. § 103(a) Over Dower '888 (U.S. Patent No. 5,492,888) in view of Bandara et al. (DNA and Cell Biology 11: 227-231)

Claims 1-4, 12, 15, 17, 19-23, 25-26 and 45 have been rejected as being obvious over Dower '888 in view of Bandara. Applicants traverse this rejection. Reconsideration and withdrawal thereof are respectfully requested.

Dower '888

Dower '888 discloses methods of recombinantly producing soluble IL-1 receptor in a cell line, purifying this protein and further administering the protein to mice to determine suppression of immune response.

Bandara et al.

Bandara discloses a strategy of gene transfer to synoviocytes, and discusses the prospects for treatment of arthritis using these genetically engineered synoviocytes. Bandara proposes a method for introducing into the synovium genes encoding proteins with antiarthritis properties

(Abstract). Bandara further proposes an indirect approach in which synovium is removed from the joint, its synoviocytes are isolated, and the cells are transduced *in vitro*. Such genetically modified cells are subsequently transplanted back into the synovium (Abstract).

Distinctions of the present invention over the cited references

The Examiner has failed to establish *prima facie* obviousness of the present invention over the cited references. While Dower '888 discloses administering the protein form of the soluble IL-1 receptor to a mouse to reduce inflammation, Dower '888 fails to disclose or suggest introducing the soluble IL-1 receptor gene into a synovial cell and transplanting the cell to a joint space of a mammal to treat arthritis. In order to remedy this defect, Bandara is cited for its disclosure of a strategy to treat arthritis by transplanting synoviocytes that have been transduced with soluble IL-1 receptor *in vitro*. Applicants note that the Bandara reference was published some time in 1992.

The present application claims priority to U. S. Patent Application Serial No. 07/630,981, having a filing date of December 20, 1990 (now abandoned). A copy of the '981 ancestor application is enclosed for the Examiner's consideration. The effective filing date of the present application is December 20, 1990, which pre-dates the Bandara reference publication date of 1992. The '981 application discloses inserting a soluble IL-1 receptor gene into a synoviocyte *in vitro*, and further injecting the transduced synoviocyte into an arthritic joint, where the soluble IL-1 receptor protein is expressed and inflammation associated with arthritis is alleviated. See page 14, line 25 to page 16, line 8, which disclose details of how this procedure is carried out. Thus, the presently claimed subject matter flows from the '981 application and priority extends to the '981 application.

Accordingly, with the removal of the Bandara reference as applicable reference to the presently claimed invention, Dower '888 alone fails to disclose of suggest the presently claimed invention. Thus, the presently claimed invention is patentable over the cited references.

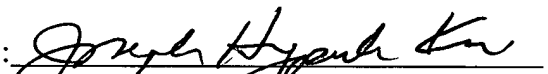
It is believed that the application is now in condition for allowance. Applicants request the Examiner to issue a notice of Allowance in due course. The Examiner is encouraged to contact the undersigned to further the prosecution of the present invention.

The Commissioner is authorized to charge JHK Law's Deposit Account No. **502486** for any fees required under 37 CFR §§ 1.16 and 1.17 that are not covered, in whole or in part, by a credit card payment enclosed herewith and to credit any overpayment to said Deposit Account No. **502486**.

Respectfully submitted,

JHK Law

Dated: March 12, 2004

By: 
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Enclosure: Specification of 07/630,981 (filing date December 20, 1990)

(Rev. 11-86)

FILING RECEIPT

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SERIAL NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTORNEY DOCKET NO.	DRWGS	TOT CL	IND CL
07/630,981	12/20/90	125	$ 435.00	109070	3	19	5

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Receipt is acknowledged of the patent application identified herein. It will be considered in its order and you will be notified as to the examination thereof. Be sure to give the U.S. SERIAL NUMBER, DATE OF FILING, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this transmittal.

Applicant(s)

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TITLE
GENE TREATMENT OF ARTHRITIS

PRELIMINARY CLASS: 514

(see reverse)

PATENT

GENE TREATMENT OF ARTHRITIS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to a method of using a gene encoding a truncated interleukin-1 receptor to resist the deleterious pathological changes associated with arthritis. More specifically, this invention provides a method wherein a gene coding for an extracellular interleukin-1 binding domain of an interleukin-1 receptor is introduced into synovial cells of a mammalian host *in vivo* for neutralizing the destructive activity of interleukin-1 upon cartilage and other soft tissues. As an alternative, the patients own cells are transduced *in vitro* and introduced back into the affected joint, using surgical transplantation procedures.

Brief Description of the Prior Art

Arthritis involves inflammation of a joint that is usually accompanied by pain and frequently changes in structure. Arthritis may result from or be associated with a number of conditions including infection, immunological disturbances, trauma and degenerative joint diseases such as, for example, osteoarthritis. The biochemistry of cartilage degradation in joints and cellular changes have received considerable investigation.

In a healthy joint, cells in cartilage (chondrocytes) and the surrounding synovium (synoviocytes) are in a resting state. In this resting state, these cells secrete basal levels of prostaglandin E_2 and various neutral proteinases, such as, for example, collagenase, gelatinase and stromelysin, with the ability to degrade cartilage. During the development of an arthritic condition, these

cells become activated. In the activated state, synoviocytes and chondrocytes synthesize and secrete large amounts of prostaglandin E_2 and neutral proteinases.

In efforts to identify pathophysiologically relevant cell activators, it has been known that the cytokine interleukin-1 activates chondrocytes and synoviocytes and induces cartilage breakdown in vitro and in vivo. Additionally, interleukin-1 is a growth factor for synoviocytes and promotes their synthesis of matrix, two properties suggesting the involvement of interleukin-1 in the synovial hypertrophy that accompanies arthritis. In contrast, interleukin-1 inhibits cartilaginous matrix synthesis by chondrocytes, thereby suppressing repair of cartilage. Interleukin-1 also induces bone resorption and thus may account for the loss of bone density seen in rheumatoid arthritis. Interleukin-1 is inflammatory, serves as a growth factor for lymphocytes, is a chemotactic factor and a possible activator of polymorphonuclear leukocytes (PMNs). When present in a sufficient concentration, interleukin-1 may cause fever, muscle wasting and sleepiness.

The major source of interleukin-1 in the joint is the synovium. Interleukin-1 is secreted by the resident synoviocytes, which are joined under inflammatory conditions by macrophages and other white blood cells.

Much attention has been devoted to the development of a class of agents identified as the "Non-Steroidal Anti-Inflammatory Drugs" (hereinafter "NSAIDs"). The NSAIDs inhibit cartilage synthesis and repair and control inflammation. The mechanism of action of the NSAIDs appears to be associated principally with the inhibition of prostaglandin synthesis in body tissues. Most of this development has involved the synthesis of better inhibitors of cyclo-

oxygenase, a key enzyme that catalyzes the formation of prostaglandin precursors (endoperoxides) from arachidonic acid. The anti-inflammatory effect of the NSAIDs is thought to be due in part to inhibition of prostaglandin synthesis and release during inflammation. Prostaglandins are also believed to play a role in modulating the rate and extent of leukocyte infiltration during inflammation. The NSAIDs include, such as, for example, acetylsalicylic acid (aspirin), fenoprofen calcium (Nalfon® Pulvules®, Dista Products Company), ibuprofen (Motrin®, The Upjohn Company), and indomethacin (Indocin®, Merck, Sharp & Dohme).

In contrast, the studies upon which the present invention is based show that production of the various neutral proteinases with the ability to degrade cartilage occurs even if prostaglandin synthesis is completely blocked.

It has been shown that genetic material can be introduced into mammalian cells by chemical or biologic means. Moreover, the introduced genetic material can be expressed so that high levels of a specific protein can be synthesized by the host cell. Cells retaining the introduced genetic material may include an antibiotic resistance gene thus providing a selectable marker for preferential growth of the transduced cell in the presence of the corresponding antibiotic. Chemical compounds for inhibiting the production of interleukin-1 are also known.

U.S. Patent No. 4,778,806 discloses a method of inhibiting the production of interleukin-1 by monocytes and/or macrophages in a human by administering through the parenteral route a 2-2'-[1,3-propan-2-onediyl-bis (thio)] bis-1 H-imidazole or a pharmaceutically acceptable salt thereof. This patent discloses a chemical compound for inhibiting the production of interleukin-1. By contrast, in

the present invention, gene therapy is employed that is capable of binding to and neutralizing interleukin-1.

U.S. Patent No. 4,780,470 discloses a method of inhibiting the production of interleukin-1 by monocytes in a human by administering a 4,5-diaryl-2 (substituted) imidazole. This patent also discloses a chemical compound for inhibiting the production of interleukin-1.

U.S. Patent No. 4,794,114 discloses a method of inhibiting the 5-lipoxygenase pathway in a human by administering a diaryl-substituted imidazole fused to a thiazole, pyrrolidine or piperidine ring or a pharmaceutically acceptable salt thereof. This patent also discloses a chemical compound for inhibiting the production of interleukin-1.

U.S. Patent No. 4,870,101 discloses a method for inhibiting the release of interleukin-1 and for alleviating interleukin-1 mediated conditions by administering an effective amount of a pharmaceutically acceptable anti-oxidant compound such as disulfiram, tetrakis [3-(2,6-di-tert-butyl-4-hydroxyphenyl) propionyloxy methyl] methane or 2,4-di-isobutyl-6-(N,N-dimethylamino methyl)-phenol. This patent discloses a chemical compound for inhibiting the release of interleukin-1.

U.S. Patent No. 4,816,436 discloses a process for the use of interleukin-1 as an anti-arthritic agent. This patent states that interleukin-1, in association with a pharmaceutical carrier, may be administered by intra-articular injection for the treatment of arthritis or inflammation. In contrast, the present invention discloses a method of using and preparing a gene that is capable of binding to and neutralizing interleukin-1 as a method of resisting arthritis.

U.S. Patent No. 4,935,343 discloses an immunoassay method for the detection of interleukin-1β that employs a monoclonal antibody that binds to interleukin-1β but does not bind to interleukin-1α. This patent discloses that the monoclonal antibody binds to interleukin-1β and blocks the binding of interleukin-1β to interleukin-1 receptors, and thus blocking the biological activity of interleukin-1β. The monoclonal antibody disclosed in this patent may be obtained by production of an immunogen through genetic engineering using recombinant DNA technology. The immunogen is injected into a mouse and thereafter spleen cells of the mouse are immortalized by fusing the spleen cells with myeloma cells. The resulting cells include the hybrid continuous cell lines (hybridomas) that may be later screened for monoclonal antibodies. This patent states that the monoclonal antibodies of the invention may be used therapeutically, such as for example, in the immunization of a patient, or the monoclonal antibodies may be bound to a toxin to form an immunotoxin or to a radioactive material or drug to form a radio pharmaceutical or pharmaceutical.

U.S. Patent No. 4,766,069 discloses a recombinant DNA cloning vehicle having a DNA sequence comprising the human interleukin-1 gene DNA sequence. This patent provides a process for preparing human interleukin-1β, and recovering the human interleukin-1β. This patent discloses use of interleukin-1 as an immunological reagent in humans because of its ability to stimulate T-cells and B-cells and increase immunoglobulin synthesis.

U.S. No. 4,396,601 discloses a method for providing mammalian hosts with additional genetic capability. This patent provides that host cells capable of regeneration are removed from the host and treated with genetic material including at least one marker which allows

for selective advantage for the host cells in which the genetic material is capable of expression and replication. This patent states that the modified host cells are then returned to the host under regenerative conditions. In the present invention, genetic material may be directly introduced (a) into host cells in vivo or (b) into synoviocytes in vitro for subsequent transplantation back into the patient's joints.

In spite of these prior art disclosures, there remains a very real and substantial need for a process wherein a gene encoding a truncated interleukin-1 receptor is used to resist the deleterious pathological changes associated with arthritis. More specifically there is a need for such a process where a gene coding for the extracellular interleukin-1 binding domain of the interleukin-1 receptor, capable of binding to and neutralizing interleukin-1 is expressed in host synovial cells in vivo.

SUMMARY OF THE INVENTION

The present invention has met the hereinbefore described need. A method of using the gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor is provided for in the present invention. This gene is capable of binding to and neutralizing interleukin-1 in vivo to substantially resist the degradation of cartilage in a mammalian host. Unlike previous pharmacological efforts, the method of this invention employs gene therapy in vivo to address the chronic debilitating effects of arthritis.

A preferred method of using the gene coding for the truncated interleukin-1 receptor of this invention involves employing recombinant techniques to generate a cell line which produces infectious retroviral particles containing the gene coding for the truncated interleukin-1 receptor. The producer cell line is generated by inserting the gene coding into a retroviral vector under the regulation of a suitable eukaryotic promoter, transfecting the retroviral vector containing the gene coding into the retroviral packaging cell line for the production of a viral particle that is capable of expressing the gene coding, and infecting the synovial cells of a mammalian host using the viral particle.

More specifically, the method of using the hereinbefore described gene involves introducing the viral particles obtained from the retroviral packaging cell line directly by intra-articular injection into a joint space of a mammalian host that is lined with synovial cells. The method of using the gene of this invention may be employed both prophylactically and in the treatment of arthritis.

In another embodiment of this invention, a method of using the hereinbefore described gene involves infecting synovial cells in culture with the viral particles and subsequently transplanting the infected synovial cells back into the joint. This method of using the gene of this invention may also be employed prophylactically and in the treatment of arthritis.

In another embodiment of this invention, a method of using the gene coding for an extracellular interleukin-1 binding domain of the interleukin-1 receptor that is capable of binding to and neutralizing interleukin 1 includes employing recombinant techniques to produce a retrovirus vector carrying two genes. The first gene encodes the

extracellular interleukin-1 binding domain of the interleukin receptor, and the second gene encodes for selectable antibiotic resistance. This method of use involves transfecting the retrovirus vector into a retrovirus packaging cell line to obtain a cell line producing infectious retroviral particles carrying the gene.

Another embodiment of this invention provides a method of preparing a gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor including synthesizing the gene by a polymerase chain reaction, introducing the amplified interleukin-1 receptor coding sequence into a retroviral vector, transfecting the retroviral vector into a retrovirus packaging cell line and collecting viral particles from the retrovirus packaging cell line.

In another embodiment of this invention, a compound for parenteral administration to a patient in a therapeutically effective amount is provided for that contains a gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor and a suitable pharmaceutical carrier.

Another embodiment of this invention provides for a compound for parenteral administration to a patient in a prophylactically effective amount that includes a gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor and a suitable pharmaceutical carrier.

It is an object of the present invention to provide a method of using <u>in vivo</u> a gene coding for the extracellular interleukin-1 binding domain of the interleukin-1 receptor that is capable of binding to and neutralizing substantially all isoforms of interleukin-1, including interleukin-1α and interleukin-1β.

It is an object of the present invention to provide a method of using a gene in vivo in a mammalian host that is capable of binding to and neutralizing substantially all isoforms of interleukin-1 and thus, substantially resist the degradation of cartilage and protect surrounding soft tissues of the joint space.

It is an object of the present invention to provide a method of using in vivo a gene coding for the extracellular interleukin-1 binding domain of the interleukin-1 receptor that is capable of binding to and neutralizing substantially all isoforms of interleukin-1 for the prevention of arthritis in patients that demonstrate a high susceptibility for developing the disease.

It is an object of the present invention to provide a method of using in vivo a gene coding for an extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and neutralizing substantially all isoforms of interleukin-1 for the treatment of patients with arthritis.

It is an object of the present invention to provide a method of using in vivo a gene or genes that address the chronic debilitating pathophysiology of arthritis.

It is a further object of the present invention to provide a compound for parenteral administration to a patient which comprises a gene encoding an extracellular interleukin-1 binding domain of the interleukin-1 receptor and a suitable pharmaceutical carrier.

These and other objects of the invention will be more fully understood from the following description of the invention, the referenced drawings attached hereto and the claims appended hereto.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the interleukin-1 binding domain amino acid arrangement.

Figure 2 shows the amino acid and nucleotide sequence of the human and mouse interleukin-1 receptors.

Figure 3 shows gene encoding a truncated interleukin-1 receptor inserted into a retroviral vector.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

As used herein, the term "patient" includes members of the animal kingdom including but not limited to human beings.

The gene and method of using the gene of this invention provide for the neutralization of interleukin-1. Interleukin-1 is a key mediator of cartilage destruction in arthritis. Interleukin-1 also causes inflammation and is a very powerful inducer of bone resorption. Many of these effects result from the ability of interleukin-1 to increase enormously the cellular synthesis of prostaglandin E_2, the neutral proteinases-- collagenase, gelatinase, and stromelysin, and plasminogen activator. The catabolic effects of interleukin-1 upon cartilage are exacerbated by its ability to suppress the synthesis of the cartilaginous matrix by chondrocytes. Interleukin-1 is present at high concentrations in synovial fluids aspirated from arthritic joints and it has been demonstrated that intra-articular injection of recombinant interleukin-1 in animals causes cartilage breakdown and inflammation.

Interleukin-1 exists as several species, each an unglycosylated polypeptide of 17,000 Daltons. Two species have previously been cloned, interleukin-1 α and interleukin-1β . The α form has a pI of approximately 5, and the β form has a pI around 7. Despite the existence of these isoforms, interleukin-1 α and interleukin-1β have

substantially identical biological properties and share a common cell surface receptor. The interleukin-1 receptor is a 80kDa (kilodalton) glycoprotein and contains an extracellular, interleukin-1 binding portion of 319 amino acids which are arranged in three immunoglobulin-like domains held together by disulfide bridges as shown in Figure 1. A 21 amino acid trans-membrane domain joins the extracellular portion to the 217 amino acid cytoplasmic domain. Figure 2 shows the amino acid and nucleotide sequence of the human and mouse interleukin-1 receptors. In Figure 2, the 21 amino acid trans-membrane region of the interleukin-1 receptor is marked by the solid line. The position of the 5' and 3' oligonucleotides for PCR are also marked by a short solid line. The lysine amino acid just 5' to the trans-membrane domain to be mutated to a stop codon is marked by a solid circle in Figure 2.

Synovium is by far the major, and perhaps the only, intra-articular source of interleukin-1 in the arthritic joint. Snyovia recovered from arthritic joints secrete high levels of interleukin-1. Both the resident synoviocytes and infiltrating blood mononuclear cells within the synovial lining produce interleukin-1.

The present invention provides a method of using in vivo a gene coding for a truncated form of the interleukin-1 receptor which retains its ability to bind interleukin-1 with high affinity but which is released extracellularly and therefore inactive in signal transduction. The binding of this truncated and modified receptor to interleukin-1 inhibits the intra-articular activity of interleukin-1.

This method of using a gene encoding the extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and

neutralizing interleukin-1 includes employing a retroviral vector carrying a truncated interleukin-1 receptor gene which encodes a truncated and soluble active form of the receptor. The expression of the novel interleukin-1 receptor gene is controlled by regulatory sequences contained within the vector that are active in eukaryotic cells. This recombinant viral vector is transfected into cell lines stably expressing the viral proteins in trans required for production of infectious virus particles carrying the recombinant vector. These viral particles are used to deliver the recombinant interleukin-1 receptor to the recipient synovial cells by direct virus infection in vivo.

The soluble human interleukin-1 receptor to be inserted into the retroviral vector may be generated by a polymerase chain reaction (PCR). An oligonucleotide complementary to the 5' leader sequence of the human interleukin-1 receptor (GCGGATCCCCTCCTAGAAGCT) and an oligonucleotide complementary to a region just upstream from the trans-membrane domain of the interleukin-1 receptor (GCGGATCCCATGTGCTACTGG) are used as primers for PCR. The primer for the region of the interleukin-1 receptor adjacent to the trans-membrane domain contains a single base change so that the lys codon at amino acid 319 (AAG) is changed to a stop codon (TAG). By inserting a translation stop codon just upstream from the transmembrane domain, a truncated form of interleukin-1 receptor that is secreted by the cell is generated. A BamHI recognition sequence (GGATCC) is added to the 5' end of the PCR primers, and following amplification, the resulting interleukin-1 receptor fragment is cloned into a BamHI site. A cDNA library from human T-cells is used as a source for the interleukin-1 receptor cDNA. To amplify the appropriate region of the interleukin-1

receptor from the cDNA library, the complementary primers are added to the DNA and 50 cycles of annealing, primer extension and denaturation are performed using a thermocycler and standard PCR reaction conditions well known by those persons skilled in the art. Following amplification of the interleukin-1 soluble receptor using the PCR process, the resulting fragment is digested with BamHI and inserted into the pLJ retroviral vector. The pLJ retroviral vector is available from A. J. Korman and R. C. Mulligan. See also Proc. Natl. Acad. Sci., Vol. 84, pp. 2150-2154 (April 1987) co-authored by Alan J. Korman, J. Daniel Frantz, Jack L. Strominger and Richard C. Mulligan. Restriction analysis was performed to determine the correct orientation of the insert.

The retrovirus vector carrying the truncated interleukin-1 receptor is transferred into the CRIP (Proc. Natl. Acad. Sci., Vol. 85, pp. 6460-6464 (1988), O. Danos and R. C. Mulligan) packaging cell line using a standard $CaPO_4$ transfection procedure and cells wherein the viral vector is stably integrated and is selected on the basis of resistance to the antibiotic G418. The viral vector containing the neomycin resistant (neo-r) gene is capable of imparting resistance of the cell line to G418. The CRIP cell line expresses the three viral proteins required for packaging the vector viral RNAs into infectious particles. Moreover, the viral particles produced by the CRIP cell line are able to efficiently infect a wide variety of mammalian cell types including human cells. All retroviral particles produced by this cell line are defective for replication but retain the ability to stably integrate into synovial cells thereby becoming an heritable trait of these cells. Virus stocks produced by this method are substantially free of

contaminating helper-virus particles and are also non-pathogenic.

More specifically, the truncated interleukin-1 gene can be inserted into a retroviral vector under the regulation of a suitable eukaryotic promoter such as the retroviral promoter already contained within the gene transfer vector, such as for example, the pLJ vector shown in Figure 3. It will be understood by those persons skilled in the art that other vectors containing different eukaryotic promoters may also be utilized to obtain a generally maximal level of interleukin-1 receptor expression. The vectors containing the truncated, and modified interleukin-1 receptor will be introduced into a retroviral packaging cell line (CRIP) by transfection and stable transformants isolated by selection for the expression of the neomycin resistance gene also carried by the pLJ vector. The CRIP cell line expresses all the proteins required for packaging of the exogenous retroviral RNA. Viral particles produced by the G418-selected CRIP cell lines will carry a recombinant retrovirus able to infect mammalian cells and stably express the interleukin-1 truncated receptor. The viral particles are used to infect synovial cells directly in vivo by injecting the virus into the joint space.

Another embodiment of this invention provides a method for using the hereinbefore described viral particles to infect in culture synovial cells obtained from the lining of the joint of a mammalian host. The advantage of the infection of synovial cells in culture is that infected cells harboring the interleukin-1 receptor retroviral construct can be selected using G418 for expression of the neomycin resistance gene. The infected synovial cells expressing the interleukin-1 receptor can then be

transplanted back into the joint by intra-articular injection. The transplanted cells will express high levels of soluble interleukin-1 receptor in the joint space thereby binding to and neutralizing substantially all isoforms of interleukin-1, including interleukin-1α and interleukin-1β.

The method used for transplantation of the synovial cells within the joint is a routine and relatively minor procedure used in the treatment of chronic inflammatory joint disease. Although synovium can be recovered from the joint during open surgery, it is now common to perform synovectomies, especially of the knee, through the arthroscope. The arthroscope is a small, hollow rod inserted into the knee via a small puncture wound. In addition to permitting the intra-articular insertion of a fibre-option system, the arthroscope allows access to surgical instruments, such that snyovial tissue can be removed arthroscopically. Such procedures can be carried out under "spinal" anesthetic and the patient allowed home the same day. In this manner sufficient synovium can be obtained from patients who will receive this gene therapy.

The synovial cells (synoviocytes) contained within the excised tissue may be aseptically recovered by enzymic digestion of the connective tissue matrix. Generally, the synovium is cut into pieces of approximately 1 millimeter diameter and digested sequentially with trypsin (0.2% w/v in Grey's Balanced Salt Solution) for 30 minutes at 37° centigrade, and collagenase (0.2% w/v in Grey's Balanced Salt Solution) for 2 hours at 37° centigrade. Cells recovered from this digestion are seeded into plastic culture dishes at a concentration of 10^4 - 10^5 cells per square centimeter with Hank's F_{12} medium supplemented with 10% foetal bovine serum and antibiotics. After 3-7 days,

the culture medium is withdrawn. Non-adherent cells such as lymphocytes are removed by washing with Grey's Balanced Salt Solution and fresh medium added. The adherent cells can now be used as they are, allowed to grow to confluency or taken through one or more subcultures. Subcultivating expands the cell number and removes non-dividing cells such as macrophages.

Following genetic manipulation of the cells thus recovered, they can be removed from the culture dish by trypsinising, scraping or other means, and made into a standard suspension. Grey's Balanced Salt Solution or other isogenic salt solutions of suitable composition, or saline solution are suitable carriers. A suspension of cells can then be injected into the recipient mammalian joint. Intra-articular injections of this type are routine and easily carried out in the doctor's office. No surgery is necessary. Very large numbers of cells can be introduced in this way and repeat injections carried out as needed.

Another embodiment of this invention is the gene produced by the hereinbefore described method of preparation. This gene carried by the retrovirus may be incorporated in a suitable pharmaceutical carrier, such as for example, buffered physiologic saline, for parenteral administration. This gene may be administered to a patient in a therapeutically effective dose. More specifically, this gene may be incorporated in a suitable pharmaceutical carrier at a therapeutically effective dose and administered by intra-articular injection.

In another embodiment of this invention, this gene may be administered to patients as a prophylactic measure to prevent the development of arthritis in those patients determined to be highly susceptible of developing this disease. More specifically, this gene carried by the

retrovirus may be incorporated in a suitable pharmaceutical carrier at a prophylactically effective dose and administered by parenteral injection, including intra-articular injection.

It will be appreciated by those persons skilled in the art that this invention provides a method of using and a method of preparing a gene encoding an extra cellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and neutralizing substantially all isoforms of interleukin-1, and thus substantially protect cartilage of a mammalian host from pathological degradation. In addition, it will be understood by those persons skilled in the art that the method of using the gene of this invention will reduce inflammation, protect soft tissues of the joint and suppress the loss of bone that occurs in patients suffering with arthritis.

It will be appreciated by those persons skilled in the art that the viral vectors employed in the hereinbefore described invention may be employed to transfect synovial cells <u>in vivo</u> or in culture, such as by direct intra-articular injection or transplantation of autologous synovial cells from the patient transduced with the retroviral vector carrying the truncated interleukin-1 receptor gene.

Whereas particular embodiments of this invention have been described above for purposes of illustration, it will be evident to those persons skilled in the art that numerous variations of the details of the present invention may be made without departing from the invention as defined in the appended claims.

We claim:

1. A method of using a gene coding for a truncated interleukin-1 receptor to resist deleterious pathological changes associated with arthritis which comprises:

employing recombinant techniques to produce a retroviral packaging cell line which contain said gene coding for said truncated interleukin-1 receptor;

inserting said gene coding for said truncated interleukin-1 receptor into a retroviral vector wherein said retroviral vector is under the regulation of a suitable eukaryotic promoter;

transfecting said retroviral vector containing said gene coding for said truncated interleukin-1 receptor into said retroviral packaging cell line for the production of a viral particle that is capable of expressing said gene coding for said truncated interleukin-1 receptor; and

infecting synovial cells of a mammalian host using said viral particle obtained from said retroviral packaging cell line.

2. The method of Claim 1 employing said gene having DNA that is capable of replication and expression in said synovial cells lining a joint space of said mammalian host.

3. The method of Claim 1 including employing said method to substantially prevent the development of arthritis in a patient having a high susceptibility of developing arthritis.

4. The method of Claim 1 including employing said method to treat an arthritic patient.

5. The method of Claim 1 including effecting the infection of said synovial cells of a mammalian host by

introducing said viral particle directly into said synovial cells lining a joint space of said mammalian host.

6. The method of Claim 5 including effecting said introduction of said viral particle by parenteral injection.

7. The method of Claim 5 including effecting said introduction of said viral particle by intra-articular injection.

8. The method of Claim 1 including effecting said infection of said synovial cells of a mammalian host by introducing said viral particles directly into synovial cells in culture to form transduced synovial cells which may be subsequently transplanted into a patient's joint.

9. The method of Claim 8 including effecting said transplantation of said transduced synovial cells into a patient's joint by employing intra-articular injection.

10. The method of Claim 1 including effecting said infection of said synovial cells of a mammalian host by introducing said viral particles to outer synovial cells.

11. A method of using a gene having a coding for a extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and neutralizing interleukin-1 which comprises:

employing recombinant techniques to produce a retrovirus vector carrying two genes wherein a first gene encodes said extracellular interleukin-1 binding domain of said interleukin-1 receptor and a second gene encodes for selectable antibiotic resistance; and

transfecting said retrovirus vector into a retrovirus packaging cell line to obtain a cell line producing nonpathogenic, replication deficient but integration competent, amphitrophic infectious retroviral particles carrying said gene.

12. The method of Claim 11 including initiating introduction of said gene by infection with said retroviral particles from said cell line directly into synovial cells lining a joint space of a mammalian host.

13. The method of Claim 11 including initiating introduction of said gene by transduction of autologous synovial cells in culture, selecting a synoviocyte cell line by treatment of cultures with antibiotic, and transplanting said selected synoviocyte cells into an affected mammalian joint.

14. The method of Claim 11 wherein effecting said introduction of said viral particles is by parenteral injection.

15. The method of Claim 11 wherein effecting said introduction of said viral particles is by intra-articular injection.

16. A method for preparing a gene encoding an extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to an neturalizing interleukin-1 which comprises:

synthesizing said gene by a polymerase chain reaction of said extracellular interleukin-1 binding domain including a signal sequence for secretion of a protein;

introducing amplified interleukin-1 receptor coding sequence into a retroviral vector;

transfecting said retroviral vector into a amphitrophic retrovirus packaging cell line; and

collecting viral particles obtained from said retrovirus packaging cell line, wherein said viral particles contain said gene.

17. The gene prepared by the process of Claim 16.

18. A compound for parenteral administration to a patient in a therapeutically effective amount which

comprises a gene encoding an extracellular interleukin-1 binding domain of an interleukin-1 receptor and a suitable pharmaceutical carrier.

19. A compound for parenteral administration to a patient in a prophylactically effective amount which comprises a gene encoding an extracellular interleukin-1 binding domain of an interleukin-1 receptor and a suitable pharmaceutical carrier.

ABSTRACT

The subject invention concerns a method of using *in vivo* a gene encoding an extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and neutralizing interleukin-1 which includes employing recombinant techniques to produce a cell line under the control of a suitable eukaryotic promoter having the gene coding for the extracellular interleukin-1 binding domain of the interleukin-1 receptor; and initiating transfection of DNA of the gene by introducing viral particles obtained from the cell line directly into synovial cells lining a joint space of a mammalian host. Alternatively, synovial cells from the patient's joint may be transduced with the retroviral vector carrying the therapeutic gene and a selectable marker for selection of only transduced cells, and the now therapeutic autologous cells may be introduced back into the joint by transplantation. Additionally, a method of preparing a gene encoding an extracellular interleukin-1 binding domain of an interleukin-1 receptor that is capable of binding to and neutralizing interleukin-1 is disclosed. A compound for parenteral administration to a patient in prophylactically or therapeutically effective amounts containing the gene of the invention and a suitable pharmaceutical carrier is also provided.

FIGURE 1

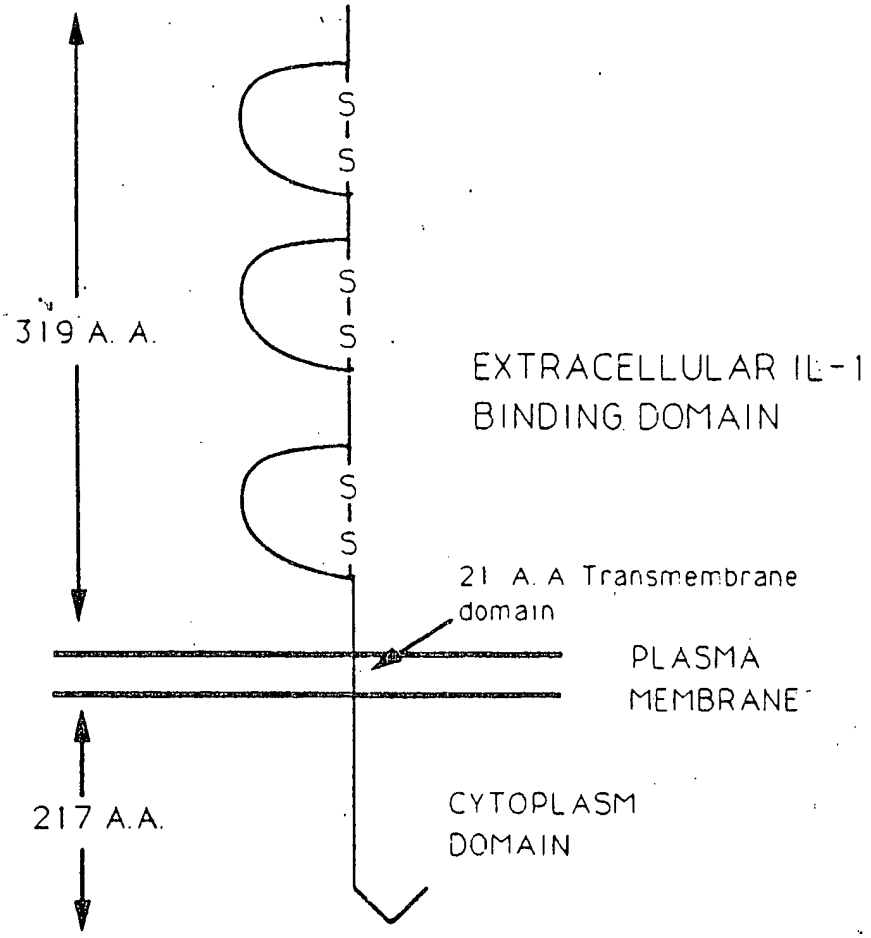


FIGURE 2

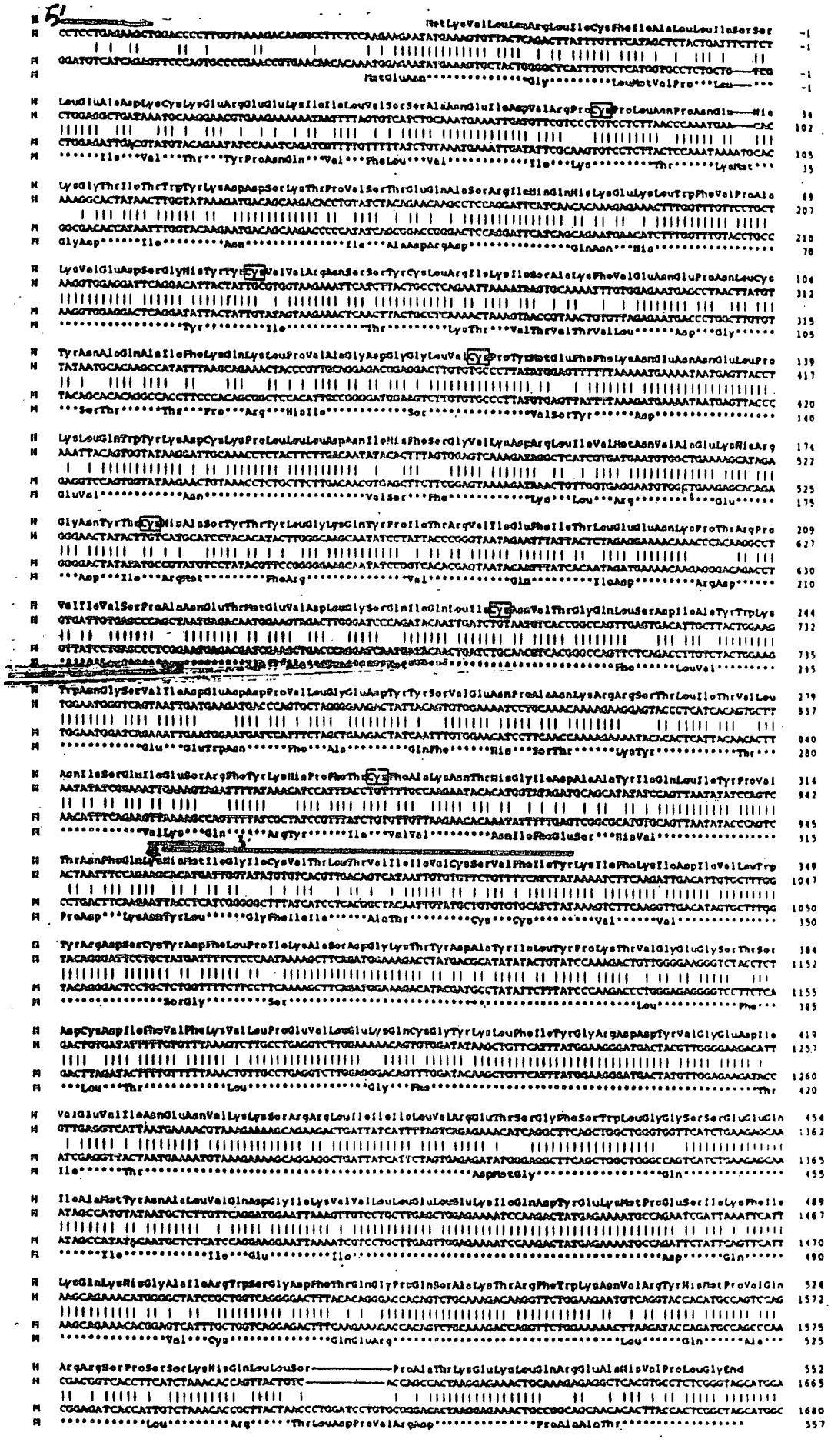
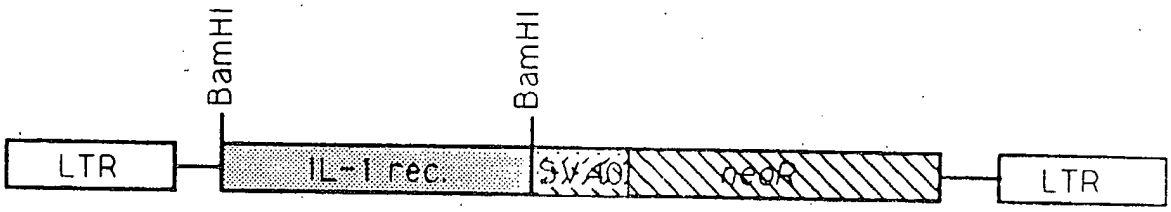


FIGURE 3

Structure of the PLJ-ILrec retroviral vector and partial restriction endonuclease map



LTR - Long Terminal Repeats - regulates viral transcription and expression of IL-1 receptor

Neo^r - bacterial gene encoding resistance to the antibiotic neomycin

SV40 - Simian Virus 40 enhancer promoter - regulates expression of the neo^r gene